Selenium Deficiency in Chicks Induced by Feeding Yeast Grown on Methanol as a Sole Dietary Protein Source

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Received August 8, 1977

Chicks fed a diet containing the yeast grown on methanol as a sole dietary protein source suffered from exudative diathesis (ED) and pancreas abnormality. Activity of their plasma glutathione peroxidase (GSH_{px}) was lowered and fragility of their erythrocytes (FE) was increased, indicating that yeast did not contain sufficient amount of either selenium (Se) or vitamin E. Lesions in pancreatic tissues, *i. e.*, necrosis of acinar areas, infiltration of eosinophilic granulocytes, and regeneration of acinar cells were recognized. Se contents in the yeast and in the diet containing the yeast were so low that they could not be detected by fluorescent method with sensitivity limit of 0.01 ppm. Addition of 0.5 ppm of Se to the diet was effective to prevent ED and pancreatic abnormality completely, to recover the activity of GSH_{px} , and to decrease FE, while addition of vitamin E was only effective to prevent ED and FE completely, but not effective to recover the activity of GSH_{px} . Addition of sodium taurocholate was not effective to prevent ED, to decrease FE, or to improve fat digestibility, indicating that vitamin E absorption was not severely damaged in the chicks fed the yeast despite histopathological change in the pancreas.

In a series of studies on nutritive value of single cell protein in this laboratory, chicks fed a diet containing 54% of a yeast grown on methanol had poor growth and died showing the symptoms characterized by capillary hemorrhage especially in peri-adipose tissue and by abdominally and thoracically located severe edema containing viscous fluid bluish-green in colour, which strongly resembled those of exudative diathesis, a typical selenium deficiency in chicks.

It has been reported that exudative diathesis (ED) was induced in chicks by feeding a diet which contained torula yeast as dietary protein source and was simultaneously deficient in selenium (Se) and vitamin E.¹⁾ Furthermore, Thompson *et al.*²⁾ reported that pancreas abnormality was also observed in the chicks fed a diet deficient in Se but sufficient in vitamin E, giving the evidence that Se is one of essential trace elements in chicks.

Accordingly, experiments had been carried

out to find the cause of death of the chicks fed the yeast. Although the diet containing 54% of the yeast induced the symptoms resembling ED in chicks, this level of the yeast resulted in too high level of dietary protein for newly-hatched chicks. Therefore, diets with lower level of the yeast were prepared in the experiments. Symptoms of the chicks fed the yeast were described, and effects of the yeast feeding and of supplementation of Se and vitamin E on performance of chicks were reported in this paper.

MATERIALS AND METHODS

Reagents. Sodium selenite, thiobarbituric acid, and 5, 5-thio-bis-2-nitrobenzoic acid were obtained from Wako Pure Chemicals Industries, Ltd., and *dl*- α -tocopheryl acetate was a powdered preparation for a medical use from Riken Bitamin-Yu Co., Ltd. 2, 3-Diamino-naphthalene was purchased from Aldorich Chemical Co., and sodium taurocholate and enterokinase preparation were obtained from Tokyo Kasei Co. Casein was a product of Merck, Darmstadt.

TABLE I. DIET COMPOSITION

	Yeast diets		Control diets	
	Yeast diet 1 %	Yeast diet 2 %	Control diet 1 %	Control diet 2 %
Yeast	44.6	35.6	—	—
Soy-bean meal	—	—	56.3	45.0
Corn starch	33.3	49.01	33.7	45.61
Lard	4.0	4.0	4.0	4.0
Cellulose	14.65	7.64	1.65	0.99
Vitamin Mix. ^{a)}	0.5	0.5	0.5	0.5
CaHPO ₄ ·2H ₂ O	—	—	2.4	2.4
CaCO ₃	2.45	2.45	0.7	0.7
NaCl	0.5	0.5	0.5	0.5
DL-Methionine	—	0.2	0.2	0.2
Trace mineral ^{b)}	—	0.1	0.05	0.1

^{a)} Does not contain vitamin E, and its composition is described in previous paper.¹⁰⁾

^{b)} Contains 67.28% of MnSO₄·4H₂O, 29.91% of FeSO₄·7H₂O, 2.43% of CuSO₄·5H₂O, 0.29% of CoSO₄·7H₂O, and 0.09% of ZnSO₄·7H₂O.

Diets. Two types each of yeast-diets and control diets were prepared, composition of which is shown in Table I. Either yeast grown on methanol, which is the yeast of *Pichia aganobii*, and is called only as yeast hereafter, or soy-bean meal was used as a sole dietary protein source. Yeast-diet 1 and control diet 1 were used in Expt. 1, of which dietary levels of metabolizable energy and crude protein were adjusted to 2.85 kcal/g and 25%, respectively. Yeast diet 2 and control diet 2 were used in Expt. 2 and 3, of which dietary levels of metabolizable energy and crude protein were adjusted to 3.0 kcal/g and 20%, respectively. Calcium phosphate was not added to yeast diets since yeast contained a lot of available phosphorus,³⁾ and ratio of Ca/P was adjusted to about 1.5/1.0 in all diets. Neither methionine nor trace mineral was added to yeast diet 1 because there was a possibility that they might contain Se as a contaminant. However, they were added to yeast diet 2 since it was confirmed by analysis of Se contents that Se could not be detected in them.

In Expt. 1, three diets, *i. e.*, control diet 1 and yeast diet 1 with and without 0.5 ppm of Se, were tested. In Expt. 2, six diets, *i. e.*, control diet 2, yeast diet 2 and yeast diet 2 with 0.5 ppm of Se, 20 or 40 ppm of *dl*- α -tocopheryl acetate, or both 0.5 ppm of Se and 20 ppm of *dl*- α -tocopheryl acetate, were tested. In Expt. 3, four diets, *i. e.*, yeast diet 2 and yeast diet 2 with supplementation of 0.5 ppm of Se or 0.5% of sodium taurocholate, or both, were tested. Sodium taurocholate was used at the expense of corn starch. To each diet was added 0.1% of chromic oxide at the expense of cellulose as an index for calculation of crude fat digestibility.

Chicks and rearing. Three experiments were con-

ducted. In these experiments, three hundred and eight newly-hatched White Leghorn male chicks were used. They were obtained from a commercial hatchery, and reared in electrically heated battery brooders for four or five weeks and fed diets and water *ad libitum*.

Expt. 1 was carried out as a preliminary experiment to elucidate whether the yeast diet 1 containing 44.6% of yeast has the ability to induce selenium deficiency in chicks. In this experiment, forty eight chicks were divided into three groups of 16 chicks each. Each group was fed each of three kinds of diets for four weeks. At the final day of four weeks chicks were sacrificed for pathological examination of Se deficiency symptoms, *i. e.*, exudative diathesis and pancreas abnormality.

In Expt. 2, two hundred chicks were divided into six groups consisting of five groups of 36 chicks each and one group of 20 chicks. To the group of 20 chicks, the yeast diet 2 with 40 ppm of vitamin E was fed, and to each of five groups, one of five diets, *i. e.*, control diet 2, yeast diet 2, and yeast diet 2 with supplemental Se or vitamin E or with both, was fed for five weeks. Body weight gain and feed intake were measured every week, and fragility of the erythrocytes of the chicks was determined at 17th day. At the final day of five weeks chicks were sacrificed by incision of the jugular vein to take blood plasma, the liver, and the pancreas. Blood plasma was kept in a refrigerator at -20°C for the later use to measure glutathione peroxidase and its protein contents. The liver was weighed and immediately homogenized to measure ascorbic acid-induced peroxidation in its mitochondria. The pancreas was weighed. Some of them were selected as the samples to be fixed in 10% formalin solution to investigate their morphological change histopathologically, or to be homogenized with 10-fold of 0.25 M sucrose solution to check its function *in vitro* by measuring casein hydrolysing activity. During the experimental period the chicks showing nutritional abnormality, *e. g.*, Se deficiency, were sacrificed when alive, and treated as described above.

In Expt. 3, sixty chicks were divided into four groups of 15 chicks each. Each group was fed each of four kinds of yeast diets with or without supplemental Se or sodium taurocholate. Body weight gain and feed intake were measured every week, and fragility of the erythrocytes were determined at 26th day. Fat digestibility at 4 week was determined by collecting fresh excreta between 27th and 28th day.

Diagnosis for exudative diathesis. Exudative diathesis was first found in the chick by greenish black color on the skin due to subcutaneous hemorrhage. The chick was watched further for a couple of days, and sacrificed before the death to confirm subcutaneous hemorrhage by observing viscous fluid bluish-green or yellow-green in color. At the end of Expt. 1 and 2,

some of the surviving chicks were sacrificed for post-mortem examination of ED and histopathological examination of the pancreas. The numbers of the chicks tested are shown in Table IV.

Fragility of the erythrocytes of the chicks. About 0.3 ml of heparinized blood was taken by pumping with an injector from the wing vein, and fragility of the erythrocytes of the chicks was determined by slightly modified method reported by Kosaka and Yoshida.⁴⁾ Incubation time was 2 hr in the estimation, not 2.5 hr reported originally.⁴⁾

Plasma glutathione peroxidase activity and protein contents. Plasma glutathione peroxidase activity was measured by the method reported by Noguchi *et al.*,⁵⁾ using 0.2 ml of plasma as enzymic source and Elmann's reagent (5, 5'-thio-bis-2-nitrobenzoic acid) to determine -SH group. Plasma protein contents were determined by Lowry's method.⁶⁾

Ascorbic acid-induced lipid peroxidation. Ascorbic acid-induced liver lipid peroxidation was determined by slightly modified Noguchi's method.⁶⁾ One ml of mitochondrial suspension and 2.5 ml of Tris-KCl buffer were used in the medium measuring lipid peroxidation, not one-half ml of mitochondrial suspension and 1.5 ml of Tris-KCl buffer reported originally.⁵⁾

Casein hydrolysing activity by pancreas homogenate in vitro. Casein hydrolysing activity by pancreas homogenate was determined by the method reported by Noguchi *et al.*,⁷⁾ using enterokinase as hydrolysis activator.

Crude fat digestibility. Crude fat in the diets and excreta was determined by extracting fat with ethyl ether twice, before and after treatment with 20% HCl solution. Chromic oxide in the diets and excreta was determined spectrophotometrically with phosphate reagent.⁸⁾ Apparent digestibility of crude fat was calculated using chromic oxide contents as an index.

Determination of selenium contents in diets and diet components. About one gram of samples were weighed exactly, and digested by Kjeldahl apparatus with 15 ml of 60% nitric acid solution for 45 min, and then with 10 ml of 60% perchloric acid solution for 2 hr. Digested samples were adjusted the acidity to pH 1.5 with 10% of ammonium hydroxide solution, and Se contents in them were determined by fluorescent method reported by Wilkie and Young,⁹⁾ using 2, 3-diaminonaphthalene as Se chelating reagent. Sensitivity limit was 0.01 ppm.

RESULT

Body weight gain and cumulative incidence

FIG. 1. Body Weight Gain and Incidence of Exudative Diathesis in the Chicks Fed the Yeast Diet.¹⁾

¹⁾ data of the chicks used in Expt. 1 are figured.

²⁾ cumulative incidence of exudative diathesis observed in the chicks fed the yeast diet.

³⁾ average body weight gain per one group.

of exudative diathesis of the chicks in Expt. 1 are presented in Fig. 1. Since no ED was observed in the chicks fed the yeast diet with supplemental Se or fed the control diet, incidence of ED in these chicks were omitted in Fig. 1 to make the figure simple and easy to understand. As shown in Fig. 1, chicks fed the yeast diet began to show the symptom resembling Se deficiency, *i. e.*, ED, between the second and the third week. This symptom was confirmed to be exudative diathesis by histopathological examination. The chicks which began to show the symptom of ED were becoming more and more reluctant to the diet, and died approximately within 10 days after ED appearance. The incidence of ED became highest between the third and fourth week.

In Table II are shown body weight gain and feed intake of the chicks used in Expt. 2. As shown in Table II and also in Fig. 1, average body weight gain of the surviving chicks fed the yeast diet remained constant after the third week, when ED was frequently observed among the chicks. There seemed to be no difference in body weight gain and feed intake among the chicks fed the yeast diet with supplemental Se, with vitamin E, and with Se plus vitamin E.

In Table III is shown preventive effect of Se and vitamin E against Se and vitamin E de-

TABLE II. BODY WEIGHT GAIN AND FEED INTAKE OF THE CHICKS FED THE YEAST-DIET

Week	Body weight gain ^{a)}					Feed intake ^{b)}				
	Yeast	Yeast (Se+)	Yeast ^{c)} (E+) g	Yeast (Se, E+)	Control	Yeast	Yeast (Se+)	Yeast ^{c)} (E+) g	Yeast (Se, E+)	Control
1st	22	21	22	21	36	44	43	43	42	56
2nd	51	49	50	51	71	113	109	108	109	159
3rd	96	95	94	95	148	216	214	212	216	297
4th	106	162	160	159	241	— ^{d)}	332	316	334	492
5th	104	245	240	242	379	— ^{d)}	491	488	490	785

^{a)} Average body weight gain per one group, and the data of Expt. 2 are used.

^{b)} Average feed intake per one group, and the data of Expt. 2 are used.

^{c)} Pooled data of the two groups fed the yeast-diet with supplemental 20, or 40 ppm of vitamin E are shown.

^{d)} Could not be measured because many chicks were dead.

TABLE III. EFFECT OF SUPPLEMENTAL SELENIUM AND VITAMIN E TO THE YEAST-DIET Se OR VITAMIN E DEFICIENCY

Measurement	Yeast	Yeast (Se+)	Yeast, E+		Yeast (Se, E+)	Control
			20 ppm	40 ppm		
ED/Total ¹⁾	30/36 ^{b)}	0/36 ^{a)}	0/36 ^{a)}	0/20 ^{a)}	0/36 ^{a)}	0/36 ^{a)}
EM/Total ¹⁾	0/36 ^{a)}	0/36 ^{a)}	0/36 ^{a)}	0/20 ^{a)}	0/36 ^{a)}	0/36 ^{a)}
Mortality (%)	83 ^{b)}	0 ^{a)}	0 ^{a)}	0 ^{a)}	0 ^{a)}	0 ^{a)}
GSH _{px} ²⁾	3.5 ± 1.8 ^{b)}	29.3 ± 1.1 ^{a)}	3.9 ± 1.4 ^{b)}	— ⁹⁾	31.0 ~ 2.4 ^{a)}	30.0 ± 1.8 ^{a)}
Fragility of erythrocytes (%) ³⁾	56 ± 15 ^{c)}	9 ± 8 ^{b)}	0 ± 0 ^{a)}	—	0 ± 0 ^{a)}	4 ± 3 ^{b)}
Plasma protein ⁴⁾	2.7 ± 0.4 ^{a)}	2.9 ± 0.3 ^{a)}	3.0 ± 0.3 ^{a)}	—	2.9 ± 0.4 ^{a)}	3.0 ± 0.3 ^{a)}
TBA value ⁵⁾	0.14 ± 0.04 ^{b)}	0.08 ± 0.03 ^{a)}	0.09 ± 0.02 ^{a)}	—	0.08 ± 0.03 ^{a)}	0.09 ± 0.03 ^{a)}
Weight of pancreas ⁶⁾	0.36 ± 0.08 ^{a)}	0.41 ± 0.08 ^{a)}	0.40 ± 0.07 ^{a)}	—	0.42 ± 0.07 ^{a)}	0.41 ± 0.05 ^{a)}
Caseinolytic activity ⁷⁾	89.1 ± 10.8 ^{a)}	91.3 ± 12.4 ^{a)}	88.9 ± 9.7 ^{a)}	—	95.1 ± 14.4 ^{a)}	91.5 ± 13.2 ^{a)}

¹⁾ Number of the chicks suffering from exudative diathesis (ED) or encephalomalacia (EM) per total number of the chicks used in the experiment.

²⁾ Mean and S.D. of the activity of plasma glutathione peroxidase of 10 chicks, and one unit is equal to the activity which causes the decrease of 1/100 μ M reduced glutathione with 0.2 ml of plasma.

³⁾ Mean and S.D. of 8 chicks, and measured at 17th day.

⁴⁾ Mean and S.D. of 15 chicks, and is shown as percent of g protein/ml plasma.

⁵⁾ Represents thiobarbituric acid value in liver lipid peroxidation, and data are shown as mean and S.D. of optical density at 530 nm using 8 chicks per one group.

⁶⁾ Mean and S.D. of 20 chicks, and data are shown as g/100g of body weight.

⁷⁾ Data are shown as mg of hydrolysed casein protein/15 min/mg of protein of pancreas homogenate, using 10 chicks per one group.

⁸⁾ Difference between figures with different superscript are statistically significant at 5% level by χ^2 - or Student's *t*-test.

⁹⁾ Was not measured.

iciency symptoms induced by yeast. Supplemental 0.5 ppm of Se or 20 and 40 ppm of vitamin E completely prevented ED and mortality. Activity of plasma glutathione peroxidase of the chicks fed yeast decreased significantly compared with that of the chicks fed the control diet. The activity recovered

to the same level as that of the control by supplemental Se, while did not recover by supplemental vitamin E. Effect of simultaneous supplementation of Se and vitamin E was not different significantly from that of Se only.

Although encephalomalacia, one of vitamin E deficiency symptoms in chicks, was not ob-

served in the chicks fed the yeast, fragility of the erythrocytes was significantly higher than that of the chicks fed the control diet. Fragility of the erythrocytes of the chicks fed the yeast was completely prevented by supplemental vitamin E and partially by supplemental Se.

Ascorbic acid-induced lipid peroxidation (TBA value) in the liver mitochondria of the chicks fed the yeast was higher than those of the other groups. Supplementation of either Se or vitamin E was effective to lower it.

Plasma protein contents of the chicks fed the yeast diet tended to be lowered than those of the other groups, but the difference in plasma protein contents was not significant statistically.

Weight of the pancreas of the chicks fed the yeast diet was slightly lighter than that of the others, though the difference was not significant statistically. No difference was observed in casein hydrolysing activity *in vitro* among all the groups.

In Table IV is shown the result of post-mortem examination for exudative diathesis and histopathological examination in pancreatic tissue of the chicks fed the yeast diets with or without supplementation and the control diet, representative microscopic pictures of which are shown in Fig. 2. Pancreatic lesions, *i. e.*, necrosis, infiltration eosinophilic granulocytes, and regeneration of acinar cells were recognized in the chicks fed the yeast diet. Not all of the chicks suffering from ED

TABLE IV. HISTOPATHOLOGICAL CHANGE IN PANCREATIC TISSUE OF THE CHICKS FED THE YEAST-DIET

Diet	No. of chicks	ED ^{a)}	Necrosis of acinar areas	Infiltration of E.G. ^{b)}	Regeneration of acinar cells
Exp. 1					
Yeast-diet	3	0	3	0	1
	8	8	4	4	5
Yeast-diet (Se+)	9	0	0	0	0
Control	5	0	0	0	0
Expt. 2					
Yeast-diet	3	0	2	1	2
	6	6	3	3	2
Yeast-diet (E+) 40 ppm	4	0	3	1	4
20 ppm	3	0	0	0	0
Control	5	0	0	0	0

^{a)} Exudative diathesis

^{b)} Eosinophilic granulocytes

TABLE V. EFFECT OF SODIUM TAUROCHOLATE ON SELENIUM DEFICIENCY

	Yeast	Yeast (Se+)	Yeast (T-Na+)*	Yeast (Se, T-Na+)
Gain of B.W. ¹⁾	125	157	116	162
ED/Total ²⁾	12/15 ^{b5)}	0/15 ^a	9/15 ^b	0/15 ^a
Fragility of (%) ³⁾ erythrocytes	78±6 ^b	32±17 ^a	73±16 ^b	21±14 ^a
Fat digestibility (%) ⁴⁾	79.3±3.3 ^a	79.5±2.2 ^a	81.7±3.0 ^a	81.4±2.4 ^a

¹⁾ Average body weight gain until 4 weeks from start of experiment.

²⁾ Number of the chicks suffering from exudative diathesis per total number of the chicks used in the group.

³⁾ Measured at 26th day and represents mean and S. D. of 8 chicks

⁴⁾ Measured at 4 week and represents mean and S. D. of three groups consisting of 5 or 6 chicks

⁵⁾ Difference between figures with different superscript are significant at 5% level by χ^2 - or Student's *t*-test.

* Abbreviation of sodium taurocholate.

FIG. 2-1. Pancreas of the Chicks Fed the Control Diet: normal, H-E $\times 100$.
FIG. 2-2. Pancreas of the Chicks Fed the Yeast Diet with Supplemental Se: no lesions, H-E $\times 100$.
FIG. 2-3. Pancreas of the Chicks Fed the Yeast Diet without Any Supplementation: focal necrosis of pancreatic tissue with infiltration of eosinophilic granulocytes, H-E $\times 200$.
FIG. 2-4. Pancreas of the Chicks Fed the Yeast Diet with Supplemental 40 ppm of Vitamin E: focal necrosis, H-E $\times 200$.

showed pancreatic abnormality, and in contrast some of the chicks fed the yeast diet and not suffering from ED showed pancreatic abnormality. Pancreatic lesions were not observed in the chicks fed the yeast diet with supplemental Se and the control diet, while observed in all of four chicks fed the yeast diet with 40 ppm of vitamin E, and were not observed in three chicks fed the yeast diet with 20 ppm of vitamin E.

In Table V is shown the effect of sodium taurocholate on yeast-induced Se deficiency. Supplementation of sodium taurocholate did not reduce incidence of ED or fragility of the erythrocytes, while that of Se completely prevented ED and partially fragility of the erythrocytes, as also shown in Table III. Digestibility of fat was not improved by supplementation of sodium taurocholate or Se.

TABLE VI. SELENIUM CONTENTS IN THE DIETS AND DIET COMPONENTS

Diets or diet components	Se contents ppm
Yeast ^{a)}	— ^{b)}
Yeast diet 1	—
Yeast diet 2	—
Soy-bean Meal	0.19 ± 0.02 ^{c)}
Control diet 1	0.12 ± 0.01
Control diet 2	0.09 ± 0.01

^{a)} Yeast grown on methanol.

^{b)} Not detectable with the sensitivity limit of 0.01 ppm.

^{c)} Mean and S.D. at four determinations.

In Table VI are shown Se contents in the diets and diet components. No detectable amount of Se, over 0.01 ppm, was found in the yeast grown on methanol, or in the yeast diet. On the other hand, sufficient amount of Se which satisfied Se requirement for chicks,¹⁰⁾ more than 0.06 ppm, was detected in the control diets.

DISCUSSION

Exudative diathesis and pancreatic abnormality, well-known symptoms of Se deficiency, were induced in chicks by feeding the semi-

purified diet containing the yeast grown on methanol as a sole dietary protein source, which were accompanied by lowering of the activity of glutathione peroxidase in blood plasma, a marker enzyme for Se contents in animals.¹¹⁾ In consistence with these observations, Se contents in the yeast was very low, below 0.01 ppm, and did not satisfy Se requirement for chicks.¹⁰⁾ Very low contents of Se in the yeast may be attributable to Se contents in the medium on which the yeast grew, and this is the point to be further studied.

Furthermore, it is suggested that the yeast diet 2 was deficient not only in Se but also in vitamin E, since fragility of the erythrocytes was still observed in the chicks fed the diet with addition of Se alone. The findings that TBA value *in vitro* was lowered and ED *in vivo* was prevented by supplemental vitamin E also indicated the deficiency of vitamin E in the diet. The latter findings agreed with the report of Dam.¹²⁾

Since pancreatic abnormality was recognized histopathologically in the chicks fed the yeast diet, it was expected that pancreatic function would have been severely damaged by yeast feeding. Thompson and Scott^{2,13)} and Noguchi *et al.*⁷⁾ reported that the chicks fed the purified amino acid diet containing less than 0.005 ppm of Se suffered severe pancreatic abnormality, *i. e.*, pancreas atrophy or fibrosis, resulting in impaired fat absorption including poor absorption of vitamin E and in high mortality. Poor absorption was reported to be improved by addition of bile acid,¹³⁾ while mortality of the chicks was not prevented by addition of even 200 ppm of *dl*- α -tocopheryl acetate.²⁾

However, in contrary to the expectation, in the chicks fed the yeast diet fat digestibility was normal and casein hydrolysing activity *in vitro* by pancreas homogenate were normal, and supplementation of sodium taurocholate was not effective to decrease fragility of the erythrocytes or ED incidence, and to increase fat digestibility, indicating vitamin E absorption was not impaired. Moreover, supplemental 40 ppm of vitamin E completely pre-

vented the mortality in the chicks fed the yeast diet despite histopathological change in their pancreas. Therefore, in this experiment, the chicks might not have been in such severe Se deficiency as reported previously.^{2,7,13)}

The difference between the observations in this experiment and in the literatures^{2,7,13)} on fat absorption function and effectiveness of supplemental vitamin E may be attributable to that in the Se contents of the chicks and diets. As described above, the chicks were obtained from the commercial hatchery, and the diet was a semi-purified diet containing the yeast as a sole dietary protein source in this experiment, while it was reported that the chicks were obtained from the hen fed the diet deficient in Se and vitamin E, and the diet was the purified crystalline amino acid diet in the works by Thompson and Scott, and Noguchi *et al.*

Hemolysis was completely prevented by vitamin E and partially by Se, in agreement with the report by Rotruck *et al.*¹⁴⁾ It has been reported that Se could not prevent encephalomalacia, one of vitamin E deficiency symptoms¹⁵⁾ in chicks. Partial effect of Se on hemolysis, however, suggests that Se has a slight sparing action for vitamin E, which may be through recovering of the activity of glutathione peroxidase in plasma.

Although two types of yeast-diets, *i. e.*, yeast diet 1 and yeast diet 2, differed in their composition and also in the dietary levels of metabolizable energy and crude protein, neither of both diets contained detectable Se contents with the sensitivity limit of 0.01 ppm, and Se

deficiency was induced by both types of diets. Therefore, it is agreeable that Se deficiency induced by the yeast diet is due to very low contents of Se, and that either dietary composition or dietary levels of metabolizable energy and crude protein have little effect to the deficiency.

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